SPECTRUM

The Creative Approach to Bioscience

Alanine aminotransferase (ALT/GPT) Colorimetric

REF: 264 001 (2 x 50 ml) 100 test
REF: 264 002 (2 x 100 ml) 200 test

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		
REF	Catalogue Number		Manufactured by
	Consult instructions for use		(Xi) - Irritant
	Temperature Limitation		

Intended Use

Spectrum ALT reagent is intended for the in-vitro quantitative, diagnostic determination of ALT in human serum.

Background

The enzyme alanine aminotransferase (ALT) is widely distributed with high concentrations in liver and to a lesser extent in kidneys, heart, skeletal muscles, pancreas and lungs. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver and chronic alcohol abuse. ALT is only slightly elevated in patients who have an uncomplicated myocardial infarction. Although both AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver specific enzyme. Moreover,elevations of ALT activity persist longer than elevations of AST activity.

Method

ALT – (colorimetric method).

Assay Principle

The reaction involved in the assay system is as follows:
The amino group is enzymatically transferred by ALT present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate.

L-Alanine + 2-Oxoglutarate $\xrightarrow{ALT}$ Pyruvate + L-Glutamate

ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Reagents

Reagent 1 (R1 Buffer)

Phosphate buffer	100 mmol/L
DL- Alanine	200 mmol/L
2 – Oxoglutarate	6 mmol/L
Sodium Azide	12 mmol/L

Reagent 2 (R2)

2,4-dinitrophenylhydrazine	2.0 mmo/L

(C)-Corrosive contains caustic material.
R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.
For further information, refer to the Alanine aminotransferase reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
Reagent (R1) contains sodium azide which may react with copper or lead plumbing.

Additional Reagent

Sodium hydroxide 0.4 mol/L.

Reagent Preparation, Storage and Stability

The reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 – 8 ^{o}C. Once opened, the reagent is stable for 6 months at the specified tempertature.

Deterioration

Do not use the ALT regents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

Use only non haemolyzed serum. The biological half-life of ALT in serum is 47 hours. The only acceptable anticoagulants are heparin and EDTA
Stability: 3 days at 15 - 25 ^{o}C or 7 days
at either 4 - 8 ^{o}C or at -20 ^{o}C

System Parameters

Wavelength	546 nm (530-550 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample: Reagent Ratio	1 : 60
Temperature	37 ^{o}C and 20 – 25 ^{o}C
Zero adjustment	Reagent or Sample blank
Sensitivity	4 U/L
Linearity	94 U/L
Reagent Blank Limits	Low 0.20 AU High 0.3 AU

Procedure

1. Measurement against Reagent Blank

Pipette into test tubes

	Reagent blank	Sample
R1(buffer)	0.5 ml	0.5 ml
Sample	------	100 µl
Distilled water	100 µl	------
Mix and incubate for exactly 30 minutes at 37 ^{o}C		
R2	0.5 ml	0.5 ml
Mix and incubate for exactly 20 minutes at 20 – 25 ^{o}C		
Sodium hydroxide	5.0 ml	5.0 ml

Mix and measure absorbance of specimen against reagent blank at 546 nm after 5 minutes.

2. Measurement against Sample Blank

Pipette into test tubes

	Sample blank	Sample
R1(buffer)	0.5 ml	0.5 ml
Sample	-----	100 µl
Mix and incubate for exactly 30 minutes at 37 ^{o}C		
R2	0.5 ml	0.5 ml
Sample	100 µl	-----
Mix and incubate for exactly 20 minutes at 20 – 25 ^{o}C		
Sodium hydroxide	5.0 ml	5.0 ml

Mix and measure absorbance of specimen against sample blank at 546 nm after 5 minutes.

Calculation

The ALT activity in the serum can be determined from the following table:

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

Performance Characterstics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	7	32
SD	0.35	0.60
CV%	3.76	1.58

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	8	35
SD	0.25	0.80
CV%	2.63	2.07

Methods Comparison

A comparison between Spectrum ALT reagent and a commercial reagent of the same methodology was performed on 200 human sera. A correlation of 0.978 was obtained.

Quality Control

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run.

Sensitivity

When run as recommended, the sensitivity of this assay is 4 U/L.

Linearity

The assay is linear up to 94 U/L. If the absorbance exceeds 0.5 at 546 nm (ALT 94 U/L) samples should be diluted 1 + 9 using sodium chloride and repeat the assay (result × 10).

Interfering Substances

Haemolysis
Erythrocyte contamination may elevate results, since ALT activities in erythrocytes are three to five times than those in normal sera.

Icterus
No significant interference.

Lipemia
Lipemic specimens may cause high absorbance flagging. Diluted sample is recommended.

Note
High concentration of aldehydes, ketones, or oxo-acids in some sera may cause false high transaminase levels. Measurement aganist a serum blank instead of a reagent blank avoids the risk of finding such artifacts.

Expected Values

Serum : up to 12 U/L.

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

4 – 94 U/L.

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Henry RJ et al. Am J Clin Path 1960 :34:381.
2. Reitman S and Frankel S . Am . J.Clin.Path., 1975 ;28;65.
3. Sherwin JE. Liver function. In:kaplan LA, Pesce AJ, eds. Clinical chemistry, theory, analysis, and correlation. St louis:Mosby;1984:420-438.
4. Young DS. Effects of drugs on clinical laboratory tests. Third edition. 1990 :3:6-12.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
264 001	2 x 50 ml
264 002	2 x 100 ml

Egyptian Co for Biotechnology - Spectrum Diagnostics (S.A.E)
Obour city industrial area. block 20008 piece 19 A. Cairo. Egypt.
Tel: +202 4665 1848 - Fax: +202 4665 1847
www.spectrum-diagnostics.com
E-mail:info@spectrum-diagnostics.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

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